

**Supplementary table 1:** Expected and measured masses of the different proteins detected in this study.

Subunit/complex	Expected mass (Da)	Measured mass (Da)	Mass difference (-Da)	Mass difference (%)
Beta (F <sub>o</sub> F <sub>1</sub> )	50,194	50,345±42	148	0.29
CytBDx*	108,267	108,141±25	126	0.11
BamABCDE+3CDL*	206,832	206,619±31	213	0.1
BamABCDE <sub>2</sub> *	213,471	213,868±40	397	0.18
Unknown		380,147±21		
AcrA <sub>1</sub> B <sub>3</sub> Z <sub>2</sub> tolC <sub>1</sub> *	444,973	445,155±46	182	0.04
F <sub>o(c11)</sub> F <sub>1</sub> +SecYEG*	611,785 (no nucleotides) 613,311±28*	613,798±15 (includes 4 nucleotides, also measured in *)	2,013	0.32/0.08
FapF**	45,039	45,104±3		
FapF <sub>3</sub> **	135,117	135,424±1		
FapD	23,313	23,313±8 Processed correctly 22,488±8 Self-processed 24,596±10 Misprocessed See supplementary figure 3	0	0
FapC	22,558			
FapE <sup>39</sup> lacking portion of N-terminus**	17,658			

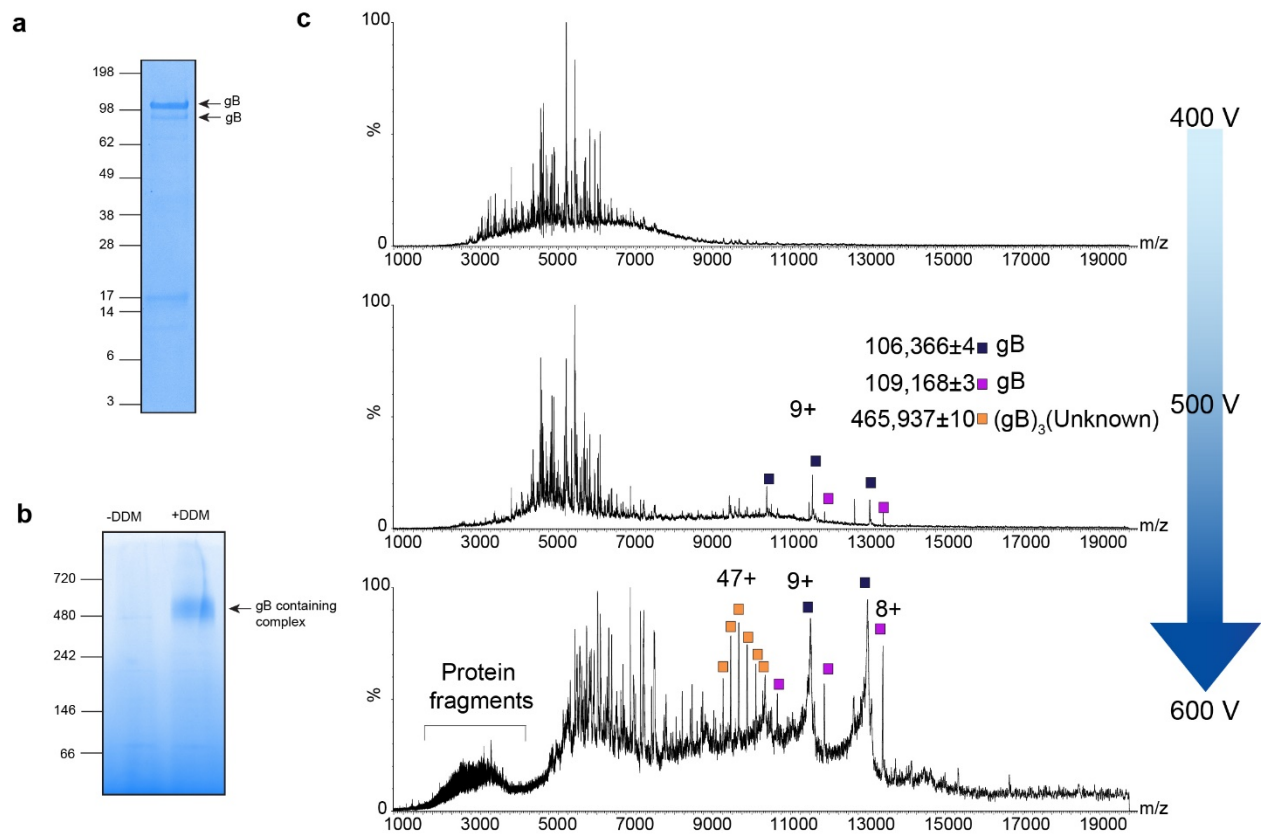
FapF <sub>3</sub> +D	158,737	158,706±5	31	0.02
FapF <sub>3</sub> +D+C	181,295	181,260±10	35	0.019
FapF <sub>3</sub> +C+D+E	198,953	199,107±15	154	0.077
Purified RsaA N1#	21,903	21,896±9	7	0.03
Purified RsaA N2#	22,035	22,033±0.4	2	0.009
Purified RsaA N3#	25,319	25,318±0.04	1	0.004
Purified RsaA C1	70,620	70,627±2	7	0.01
Purified RsaA C2	73,002	73,044±4	42	0.06
Purified RsaA C3	73,476	73,464±10	12	0.02
Purified RsaA C4	74,470	74,460±18	10	0.01
Purified RsaA #	98,002	98,515±38	513	0.52
Purified RsaA <sub>2</sub> #	196,004	197,245±64	1,241	0.63
Purified RsaA <sub>3</sub> #	294,006	295,894±93	1,888	0.64
S-layer RsaA#	98,002	98,425±43	423	0.43
S-layer RsaA <sub>2</sub> #	196,004	196,858±91	854	0.44
S-layer RsaA <sub>3</sub> #	294,006	295,144±78	1,138	0.39

**\*Masses previously reported in<sup>1</sup>.**

**\*\*Masses previously measured in<sup>42</sup>.**

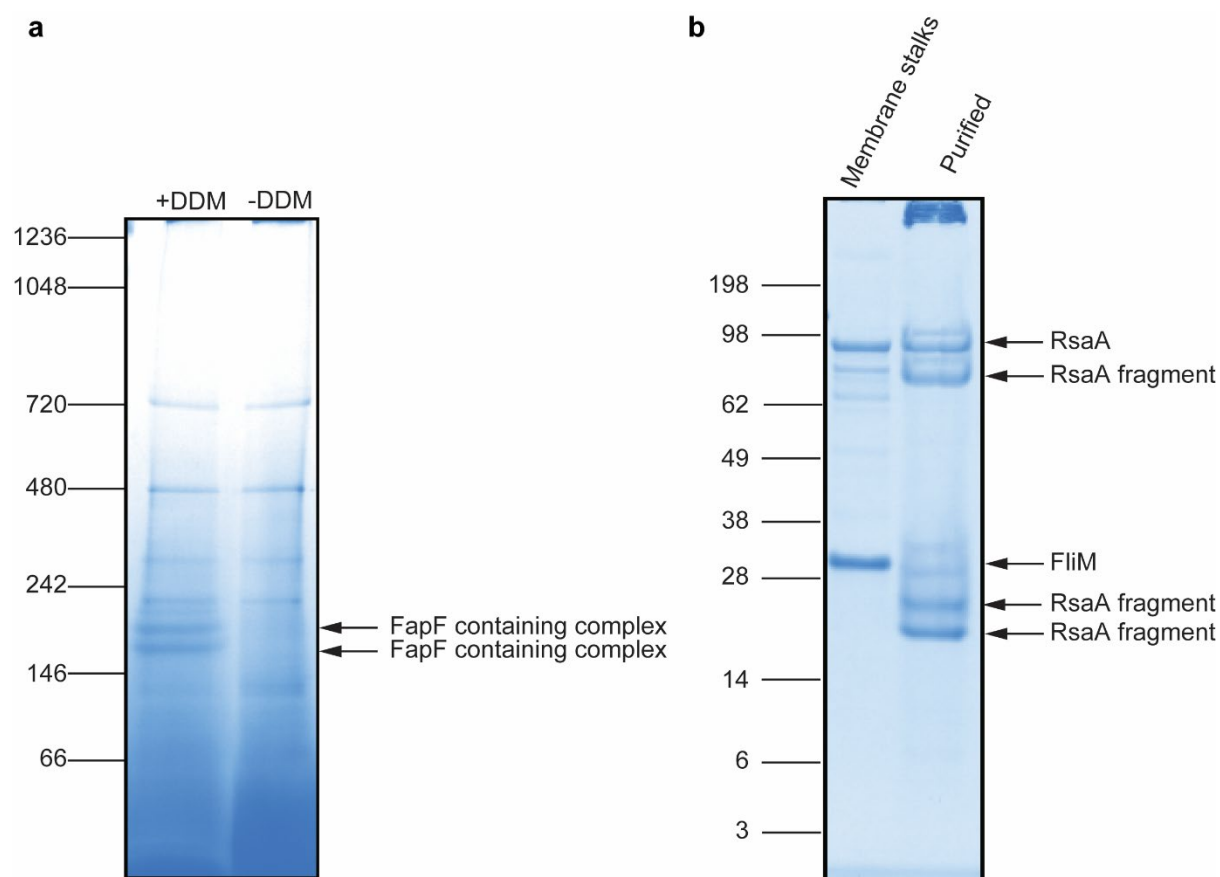
**#Initial methionine removal was considered when we calculated the expected masses of RsaA.**

The standard deviation in fitting the identified peaks to the charge series is given as the ± error in the measured mass for a given single measurement. This is the error in the fit and not the error in the mass measurement, which is likely an order of magnitude higher due to solvation/adduct effects, heterogeneous post-translational modifications, etc. Hence, the error gives a rough measure of the accuracy of peak assignment, which is impacted by the broadness, symmetry and signal:noise of each peak.

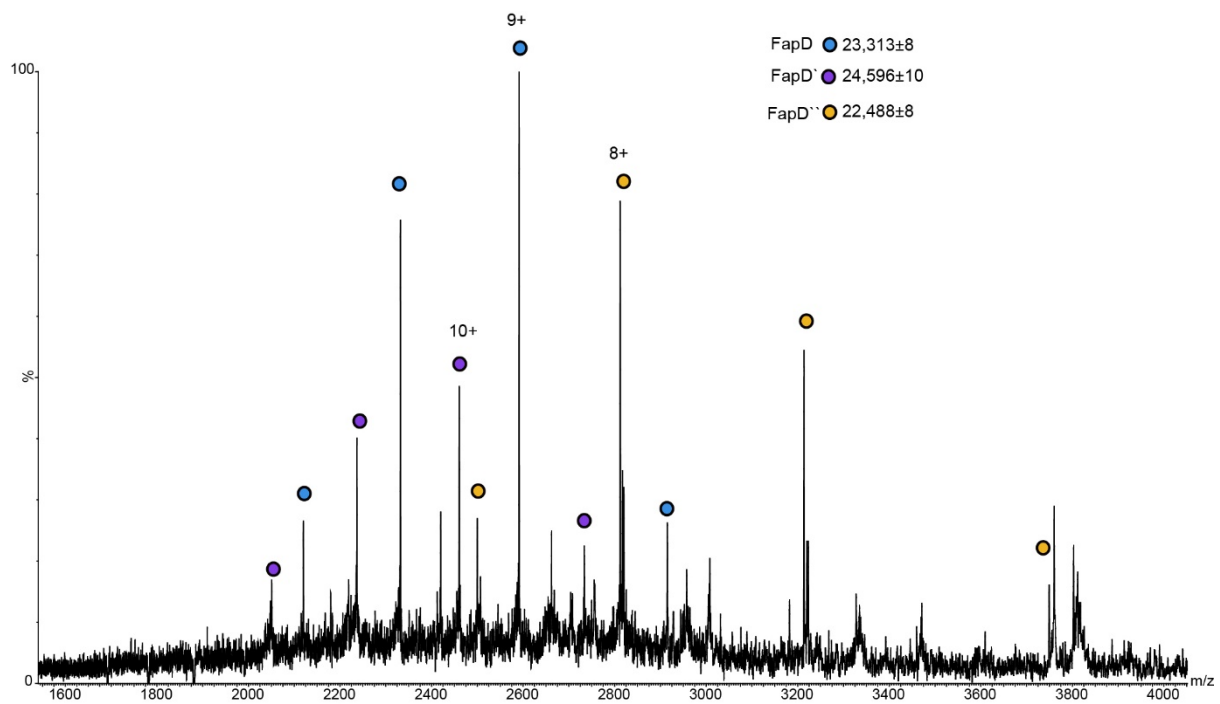


**Supplementary figure 1: Large protein complexes containing gB eject in high-energy conditions.**

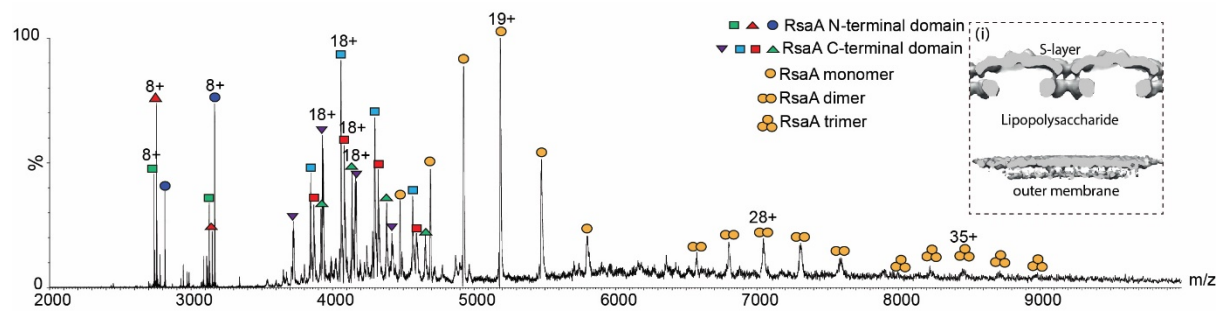
**(a)** SDS-gel of MPEEV containing the protein gB. **(B)** Native gel of the vesicles shows only one protein complex at ~480kDa. **(c)** The vesicles are first analysed at 400 V (source fragmentation 300 V, 100 V HCD) As the energy is increased, more proteins are released from the vesicle, at 500 V (additional 100 V in the HCD cell) monomers of gB start appearing, whereas at 600 V (additional 200 V in the HCD cell) a 465 kD complex appears. 2 biological repeats and 4 overall injections were performed.



**Supplementary figure 2: BN and SDS PAGE of samples.** (a) BN-PAGE of *E. coli* membranes, without major outer membrane complexes, overexpressing the Fap operon with and without DDM indicates the existence of two 180-200 kDa membrane protein complexes in the presence of DDM containing Fap proteins. (b) SDS-PAGE of *C. crescentus* membrane stalks containing the S-layer or extracted S-layer protein RsaA shows degradation products following low pH extraction.



**Supplementary figure 3: Low  $m/z$  region of protein from the FapF operon purified in detergent shows different FapD species dissociating from the FapF<sub>3</sub>+FapD complex.** High energy disruption of the FapF<sub>3</sub>+FapD complexes at 400 V induces their dissociation and reveals the co-existence of three different FapD species- a correctly processed FapD at 23,313 Da, an incompletely processed FapD' at 24,596 Da and a self-processed FapD'' with a mass of 22,488 Da. Spectrum is a representative from 2 biological repeats.



**Supplementary figure 4: Mass spectrum of low-pH extracted RsaA.** Mass spectrum of solution extracted RsaA shows the presence of RsaA monomer and dimer with only a very low population of trimer. Inset (i) shows the organization of the S-layer associated with LPS and tethered to the outer membrane. The spectrum was acquired at a capillary voltage of 1.4 kV, capillary temperature of 50 °C, with source fragmentation set to 100 V, desolvation voltage set to 0 V and HCD energy at 0 V.